Study of Root Uptake and Xylem Translocation of Cinmethylin and Related Compounds in Detopped Soybean Roots Using a Pressure Chamber Technique

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ABSTRACT

A pressure chamber technique was used to study the root uptake and xylem translocation of nonradiolabeled cinmethylin and its analogs in detopped soybean (*Glycine max*) roots. Quantifications of compounds were achieved by gas chromatography analysis using a mass spectrometry detector under selected ion monitoring. The compounds tested, with octanol-water partition coefficients (log Kow values) ranging from 0.96 to 5.3, were all nonionizable under the experimental conditions. Root efflux curves of all compounds exhibited a steady-state kinetic profile. The time required to achieve the steady state efflux concentration in the xylem sap correlated with log Kow values in a manner very similar to the root binding profile reported previously by GG Briggs et al. ([1982] Pestic Sci 13: 495-504). After reaching the steady state efflux, the concentration ratio of each compound in the xylem sap to the final concentration in the pressure chamber was taken as the transpiration stream concentration factor (TSCF). A nonlinear relationship was observed between TSCF and log Kow values. The highest TSCF value was between 0.6 to 0.8 for compounds with log Kow between 2.5 to 3.5. The range of optimal log Kow values was slightly higher than that reported earlier by Briggs et al. ([1982] Pestic Sci 13: 495-504). After taking into account the binding of the compound to soil, the apparent optimal Kow value for best root-to-shoot translocation is lowered to around 1. The relationship of root-to-shoot and phloem translocation was also discussed to promote a better understanding at the whole plant level of the uptake and translocation of a soil-applied xenobiotic.

For most agricultural chemicals, systemic distribution in the target plant is a desirable trait. For foliarly applied chemicals, systemicity requires good phloem mobility, whereas for soil applied chemicals systemicity is dependent on root uptake and xylem translocation to the shoot. Both phloem and xylem translocations of agricultural chemicals are active research areas. Recently a unified model for the phloem mobility of xenobiotics has been proposed and experimentally confirmed (12, 14). By comparison, root-to-shoot translocation via xylem is relatively less well studied, perhaps due to technical difficulties associated with root experimentations. Virtually all previous studies in this area have been entirely dependent on the availability of radiolabeled chemicals (3, 4, 10, 15-18, 20).

This paper describes the use of a root pressure chamber

technique to study the root-to-shoot translocation of cinmethylin, (+)-1-methyl-4-(1-methylethyl)-2-exo-((2-methylphenyl)methoxy)-2-oxabicyclo[2.2.1]heptane (the active ingredient of CINCH herbicide), and its analogs. The root pressure chamber technique has been very useful in studying the hydraulic and osmotic properties of root systems (7, 8). The adaptation of this technique for root-to-shoot translocation experiments on xenobiotics obviates the need for radiolabeled compounds. It also generates data pertaining to a compound's root binding and TSCF[1] simultaneously.

MATERIALS AND METHODS

Plant Materials

Hydroponically grown soybean (*Glycine max*, cultivar McCall) plants were used throughout the experiment. Seeds were germinated in vermiculite. After germination, 1-week-old seedlings were shaded to stimulate internode elongation. The shading was terminated once the internode below the cotyledonary node reached about 50 mm in length. Seedlings were transferred to 10-L stainless steel containers for hydroponic cultivation in half-strength Hoagland solution. All plants were kept in a Conviron environmental growth chamber. Approximately 2-month-old plants, between R4 and R6 early pod-fill stages (6), were used for experiments.
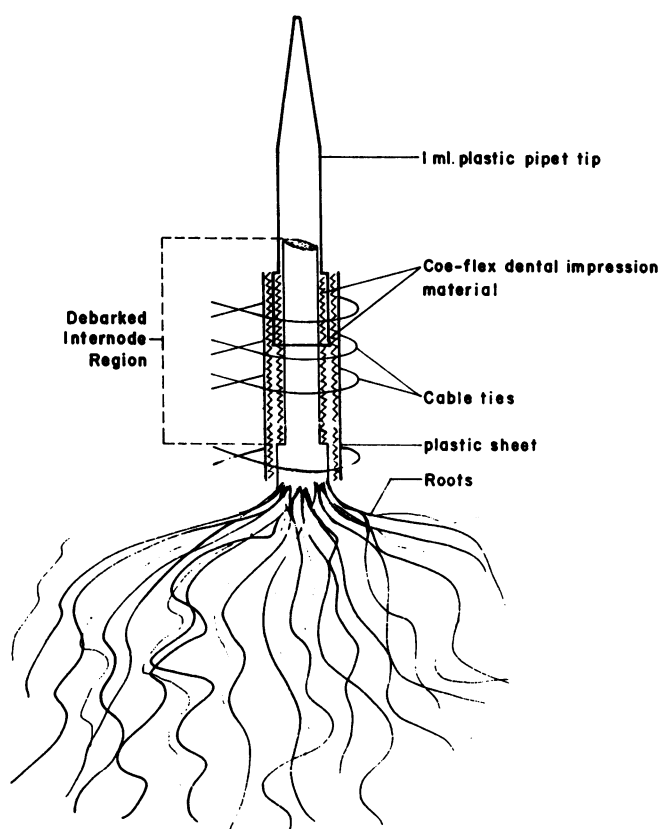
Root Pressure Chamber Technique

Plants were decapitated just below the cotyledonary node at the start of each experiment. The whole root was bathed in a half-strength Hoagland solution in the root chamber. The internode (about 50 mm long) above the root system was debarked and trimmed. It was then carefully sealed in a 1-mL plastic disposable pipet tip with Coe-flex dental impression material. A small piece of plastic film was wrapped around the dental material. It was then fastened with four cable ties so that the dental material (still soft) was tightly pressed against the internode surface. At least one cable tie (usually two) was fastened at the debarked region. The purpose of sealing with the dental material was to prevent water

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[1] Abbreviations: TSCF, transpiration stream concentration factor; log Kow, octanol-water partition coefficient; cinmethylin, (+)-1-methyl-(1-methylethyl)-2-exo-((2-methylphenyl)methoxy)-2-oxabi-cyclo[2.2.1]heptane; TSSC, time to steady-state concentration; MSD, mass selective detector.

movement between debarked surface and pipet tip, or between bark and woody center of the internode. With a proper seal, the solution pushed out of the pipet tip would be true xylem sap, uncontaminated by spurious flow-through of Hoagland solution from the root-pipet tip assembly (Fig. 1). After the dental material completely hardened, the tapered end of the pipet tip was fitted through a rubber stopper. The whole assembly (decapitated root, pipet tip, and rubber stopper) was fitted to the opening in the center of the pressure chamber lid.

The chamber was tightly sealed. Hydrostatic pressure (0.27–0.45 MPa), generated by compressed air, was applied to the root. The pressure was held constant throughout a given experiment. This dynamic pressure constancy was achieved by continuously bleeding air (at about 50 mL/min) through an exit port at the chamber head air space and replenishing the lost air via a bubbling device at the bottom of the chamber. The constant air loss and replenishing also served to supply oxygen for normal root functioning and to create a turbulence in chamber solution which helped maintain a uniform distribution for all solutes (including the injected test compounds). The temperature inside the chamber was regulated at 25 ± 0.2°C in all experiments. This was accomplished by an Omega temperature controller which used real-time chamber temperature inputs to adjust the temperature of water flowing through coiled metal tubes near the bottom of the chamber.

Figure 1. Diagram of soybean root-pipet tip assembly for pressure chamber.

Table I. *Structures of Oxabicycloalkanes and Their log Ko/w, TSCF, and TSSC Values*

Compound No.	Log Ko/w	TSCF	TSSC
			h
SD207573	0.963	0.218	0-4
SD208380	1.822	0.243	0-4
SD208213	2.523	0.552	4-8
SD98319	2.733	0.582	0-4
SD204691	3.532	0.723	8
SD205857	3.554	0.513	0-4
SD204689	3.677	0.466	12
SD96638	4.097	0.349	12
SD204690	4.197	0.520	16
SD204328	4.590	0.498	24
SD95481	4.617	0.080	20
SD208586	5.286	0.192	24

After reaching the desired pressure setting and a steady xylem sap flow, compounds of interest were injected into the chamber via a pressure-sealed port on the mid section of the chamber. For each experiment, the injection contained 10 mg of a single oxabicycloalkane compound. The volume of the Hoagland solution in the pressure chamber was 17 L at the beginning of each experiment. Xylem sap was collected by a fraction collector. Fractions were combined into 4-h samples (400–650 mL) for analysis. Two replicates were run for most compounds.
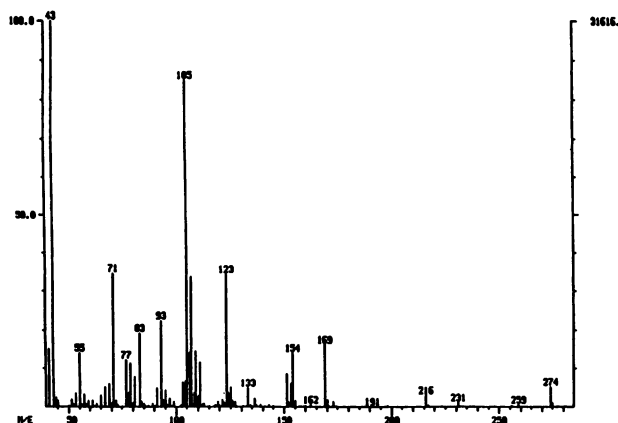
Figure 2. Mass spectrum of cinmethylin (SD95481).

Oxabicycloalkane Chemicals

Oxabicycloalkane compounds used in this study were: cinmethylin (SD95481), SD96638, SD208380, SD205857, SD208213, SD204689, SD208586, SD207573, SD98319, SD204328, SD204690, SD204691. These compounds have a wide range of lipophilicity, and their metabolism in plant tissues within the experimental period in this study (24 h) is negligible (unpublished data). Also, these chemicals are non-ionized under the experimental conditions. The structures of these compounds and their measured or calculated log Kow values are listed in Table I. The calculations of log Kows were done using a CLOG P program developed from the Pomona College data base.

Analysis of Oxabicycloalkane Compounds in Xylem Sap

Each oxabicycloalkane compound was tested separately. From each 4-h xylem sap sample, 200 or 300 mL aliquots were taken and partitioned with 50 mL of ethyl acetate three times. The combined ethyl acetate fractions were concentrated on a steam bath using Snyder distillation columns. The ethyl acetate solution was adjusted to a known volume for analysis.

A Hewlett-Packard 5970A Mass Selective Detector/5790 GC Instrument was used for analysis. A 30 m × 0.25 mm i.d., SE-54 fused silica capillary column run in the temperature program mode from 200 to 250°C was used with a split ratio of about 10:1. The injector temperature was set at 250°C. The MSD electron energy was set at 70 eV and the electron multiplier at 1800 to 2200 V.

The peakfinder software for the MSD was used to obtain full spectrum data for each compound analyzed. The spectrum of each compound was examined and the most abundant m/z ion (base peak) was recorded. Such a typical mass spectrum for cinmethylin is shown in Figure 2. Once the base peak was determined for each compound, the selected ion monitoring software (SIMS) for the MSD was used to set up to the nearest 0.1 amu on this mass (m/z) for the analysis of each compound, using a calibration curve of three standards and relating nanograms of analyte to milligrams of each sample analyzed. By using the SIMS program all the pertinent

GC and mass spectrum data were set before each GC analysis run. The GC conditions varied for each compound using a programmed column temperature run based on the retention time of each compound. Following each GC/MSD run, the integrated output was tabulated and calculated to give the concentration in the xylem sap. Data like these were used for calculations and graphs in the following sections.

Calculation of Transpirational Stream Concentration Factor

The efficiency of translocation of a chemical to shoot from root uptake was described by its TSCF as defined by Shone and Wood (17). At the end of each experiment, the concentration of compound remaining in the Hoagland solution in the root chamber was determined. This value was used for the calculation of TSCF according to the following equation:

$$TSCF = \frac{\substack{\text{concentration in xylem sap} \\ \text{at steady-state efflux}}}{\substack{\text{concentration in external} \\ \text{solution at end of exp}}}.$$

RESULTS AND DISCUSSION

Root xylem efflux curves of all compounds are shown in Figures 3 and 4. All curves exhibit a steady state kinetic profile analogous to those obtained in a stem perfusion experiment (2) and an uptake experiment on underground plant tissues (15). A steady state concentration was reached after an initial 'breakthrough' phase of varying time. Such curves resemble the elution profile for column chromatography. The time required to achieve a steady state efflux concentration can be assumed to be primarily determined by the affinity of compounds towards lipophilic plant tissue components. As a first
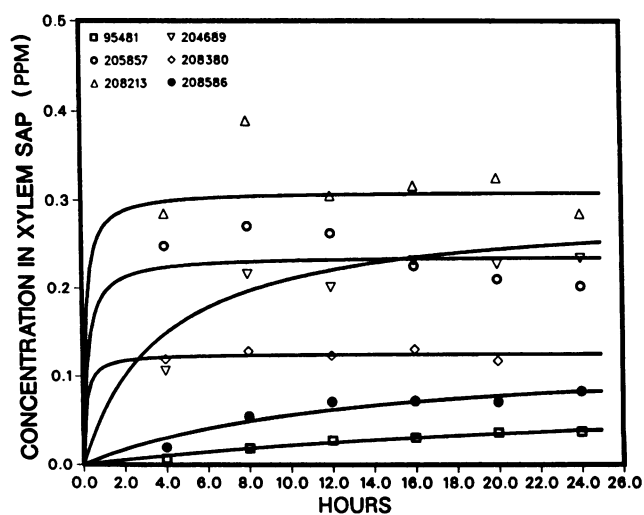

Figure 3. Root efflux curves of SD95481, SD204689, SD205857, SD208213, SD208380, SD208586.
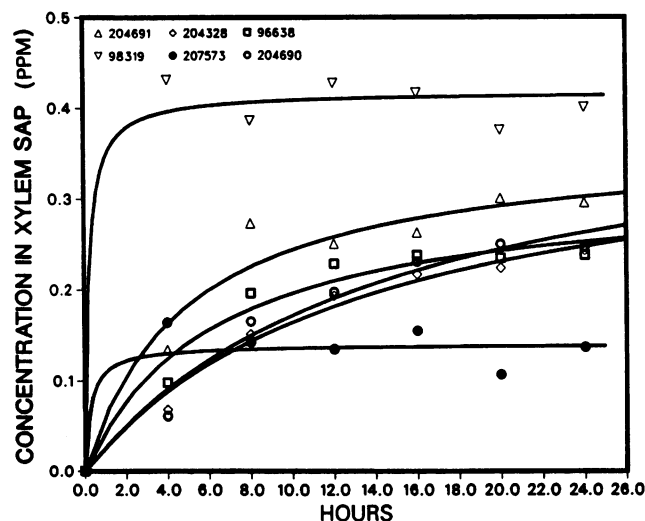
$$PPM = \frac{a \times Time}{b \times Time + 1}$$

Figure 4. Root efflux curves of SD96638, SD98319, SD204328, SD204690, SD204691, SD207573.

$$PPM = \frac{a \times Time}{b \times Time + 1}$$

approximation, the length of this breakthrough time may be regarded as positively correlated to a compound's root binding.

Figure 5 shows the relationship between different compounds' breakthrough times and their log Kow values. The curve closely resembles the one relating root concentration factor and pesticides' log Kow values in several different studies (3). The breakthrough time for compounds with log Kow values greater than 2.5 appears to increase linearly with log Kow (Fig. 5). However, the breakthrough times of compounds with log Kow values between 0 and 2.5 are similar. This region of the curve probably reflects the equilibration of compounds in the external solution with tissue water in the root (3) and not the binding of compounds to solid root matrix materials.

Many cell layers lie between the root periphery and root xylem vessels which are located in the center of the root. There are two major pathways for the transport of a molecule from the root periphery to xylem vessels: (a) apoplastic, *i.e.* via the cell wall space and only across cell membranes twice at the endodermis region (to get past the casparian strip barrier); (b) symplastic, *i.e.* across cell membranes and tonoplast membranes many times (21). The apoplastic pathway is favored by less lipophilic compounds, whereas the symplastic pathway is favored by more lipophilic compounds. The frequency of membrane crossing depends on the affinity of the compound towards the membrane which, in turn, is dictated by its log Kow value.

Compounds with low log Kow values may pass from the root periphery to xylem predominantly through the apoplastic pathway with little retention by root tissue, thus reaching a steady state efflux very quickly (Table I; Fig. 5). Compounds with higher lipophilicity would tend to take the more lipophilic symplastic pathway, and be partitioned into root tissues along the whole pathway. It would take much longer time to

saturate all binding sites in the root before a steady-state efflux is reached (Table I; Fig. 5).

When the TSCF values of various compounds are plotted against the log Kow values, a nonlinear relationship appears (Fig. 6). All TSCF values are less than unity, strongly suggesting passive uptake and translocation in the plant (3, 16). The highest values of TSCF are between 0.6 and 0.8, a range very close to those previously reported for very different chemicals (3). Compounds with log Kow values between 2.5 to 3.5 gave the highest TSCF values (Fig. 6). The relationship between TSCF and log Kow cannot be adequately explained by a single membrane permeation model in which the rate of penetration increases monotonously with log Kow before approaching a constant value (5). The simplest explanation may be that TSCF is dependent upon two factors: membrane permeability and partitioning into xylem sap. Compounds with log Kow values greater than 3.5 have excellent membrane permeation, but their partitioning into the xylem sap is greatly curtailed (due to low water solubility) resulting in a net progressive decrease of the TSCF (Fig. 6). At the other extreme, compounds with very low log Kow values can never accumulate to high concentrations in the root tissue. Due to the lack of lipophilicity, they also cross cell membranes more slowly at the Casparian strip barrier. Both factors would make the partitioning of these compounds into xylem sap less than optimal.
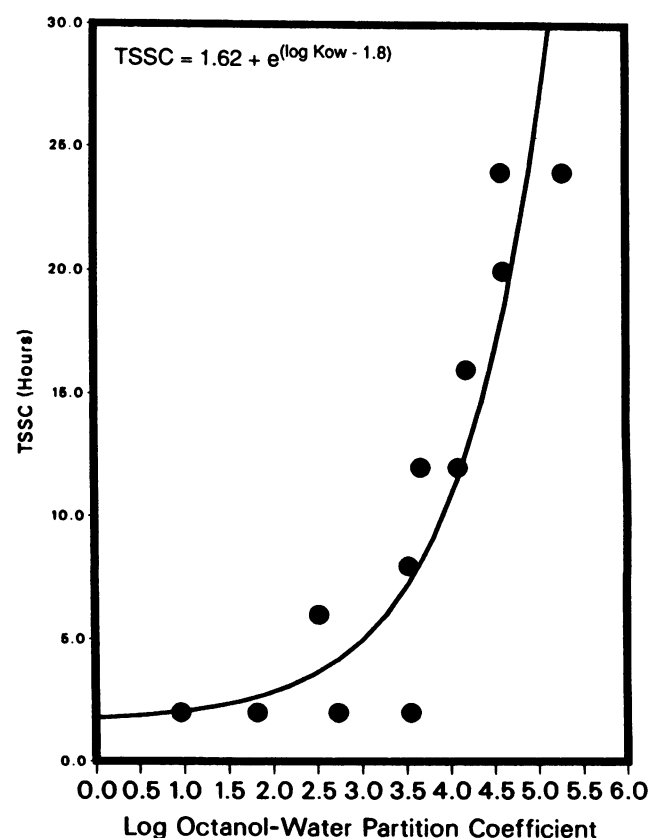

Figure 5. Relationship between time to steady-state concentration (TSSC) and compounds' log Kow values.

It is known that the phloem delivery of a xenobiotic can be strongly influenced by many plant parameters (14, 19). The differences in plant species, plant size, and experimental conditions between this study and the one by Briggs et al. (3) may account for the small difference in the observed optimal log Kow values for TSCF.

Figure 6 shows that compounds with log Kow values between 2.5 to 3.5 would possess the most desirable root-to-shoot translocation property. But when the soil-binding factor is also considered, the overall optimum log Kow range for root-to-shoot translocation would be lowered due to the linear correlation of soil binding and log Kow of the compound (1). Figure 7 illustrates the influence of soil binding on the apparent root-to-shoot translocatability from soil at 0.1 MPa moisture when equal amount of xenobiotic is applied on the dry soil weight basis. The curves are computed using the linear correlation equation of soil binding and log Kow reported by Kenaga and Goring (13). The optimal log Kow is lowered by about 2 units from the optimum of TSCF curve.

When the range of optimal log Kow values for phloem mobility (14) and that of the TSCF (Fig. 6) are compared, it is clear that the two do not overlap. This implies that it may be very difficult, or impossible, to create a xenobiotic with both excellent phloem mobility and root-to-shoot translocation properties. However, when the soil binding factor is taken into consideration (Fig. 7), compounds with log Kow of 0 to 1.0 should possess good systemicity regardless of whether they are applied to the soil or to the foliage.

It should be pointed out that the root pathway to xylem for the foliarly applied ambimobile xenobiotics (11, 19) differs considerably from that of a soil applied xenobiotic. Following a foliar application, the phloem-mobile xenobiotic gets unloaded (symplastically, apoplastically, or both; [9]) from phloem in, or near, the stele of the root. The distance between the phloem unloading site(s) and the xylem vessel in the stele is much shorter than that between the root periphery and the xylem. The xylem mobility of ambimobile xenobiotics may
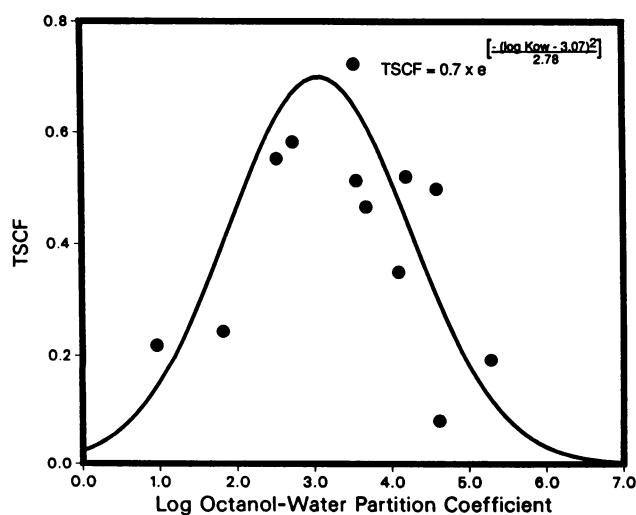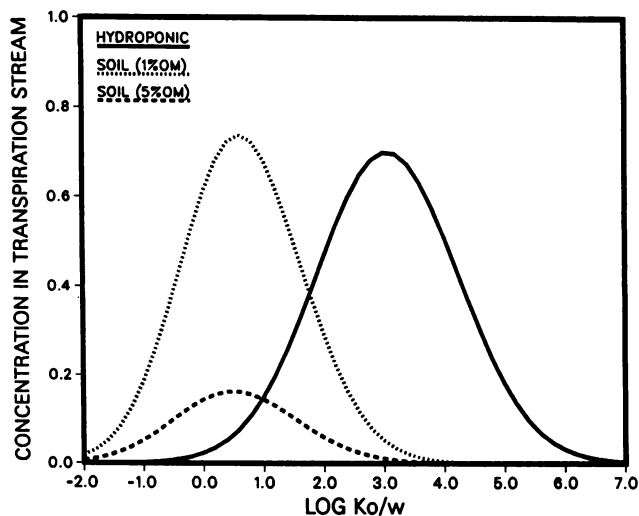

Figure 7. Effect of soil organic matter on the relationship between TSCF and compounds' log Kow values. OM, organic matter; log K_d (soil binding constant) = log (%OM/100) + 0.94(log Ko/w) − 0.2165.

only reflect this phloem-to-xylem recycling within the root stele, without having to involve Casparian strip crossing. The physicochemical property requirements on the xenobiotics for this process are likely to be less stringent than those for xylem transport from the root surface.

This study has demonstrated that the root pressure chamber is a valuable tool for root-to-shoot translocation experiments. It generates large quantities of xylem sap for compound analysis, thus obviating the necessity of radiolabeled compounds. The lack of the attached shoot eliminates the complication of compounds' effects on transpiration rate. The very similar results obtained here compared to studies using the seedling plants strongly indicate the validity of the technique. Furthermore, root efflux curves (Figs. 3 and 4) provide kinetic information on root-to-shoot translocation. The analysis of efflux curves generates both the root binding and TSCF values of compounds (Figs. 5 and 6) simultaneously. This technique appears to be well suited for studying root zone factors, such as solution pH (especially for ionizable compounds) and temperature (20) that may influence compound uptake and translocation in the xylem.

ACKNOWLEDGMENTS

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Figure 6. Relationship between TSCF and compounds' log Kow values.

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